IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 9023
STEPHENSON	Art Unit: 1642
Appl. No.: 10/528,029	
§ 371 Date: December 16, 2005	
	Examiner: Mark Halvorson
U.S. Nat'l Stage of: PCT/AU2003/001209	
IA Fd: September 16, 2003	Atty. Docket: 2381.0010000/MAC
For: **Methods for Regulating Cancer**	

Declaration Under 37 C.F.R. § 1.132

Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Madam Commissioner:

The undersigned, Sally-Anne Stephenson, declares and as follows:

1. I am the inventor of the above-captioned application.

2. I am familiar with the claims in this application as amended on June 6, 2008.

3. I have read the Office action dated September 15, 2008.

4. In the Office action, Examiner maintains the rejection of claims 1-5, 7-12 and 14-10 under 35 U.S.C. § 112, first paragraph, stating that these claims contain subject matter that was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

5. I respectfully disagree and provide evidence herein that the claims are fully enabled by the specification.

6. At Office action page 3, Examiner notes that the claims are drawn to a method for

(a) inhibiting the proliferation of a cancer cell,

(b) inducing the death of a cancer call; and

(c) treating or preventing cancer in a subject.

(d) Also, that the method of the invention comprises contacting the cells with an antibody to an epitope on EphB4. Examiner states that the claims read on a method of preventing or treating cancer in a subject.

7. Examiner looks to the specification and notes that:

(a) the specification discloses that EphB4 expression was upregulated in colon and breast cancer cells (Example 1);

(b) polyclonal antibodies to EphB4 induced cell death in breast and colon cancer cell lines (Example 3);

(c) cell death by the polyclonal antibodies is inhibited by specific EphB4 peptides (Figure 14).

8. Examiner notes that the specification does not disclose any in vivo studies on the treatment of cancer with antibodies to EphB4.

9. I am pleased to provide in vivo evidence herewith, as further evidence of the enablement of the teaching of the specification. I will first provide *in vivo* evidence of efficacy against breast cancer. Then, I will discuss how the teachings of the specification are allow the artisan of ordinary skill to practice the invention indicative against other types of cancers that involve cancerous cell that expresses EphB4 without undue experimentation.

In Vivo Studies Against Breast Cancer

10. An *in vivo* study was commissioned to determine the efficacy of the novel antibodies against the MDS-MB231 Breast Tumour in Female BALB/c Nude Mice. The study was performed for the assignee by *vivo*Pharm Pty Ltd, Level 9, 195 North Terrace, Adelaide, SA 5000 Australia.

11. An antibody was raised against an epitope within residues 220 to 230 of the human EphB4 protein. The antibody produced was a mouse monoclonal antibody produced according to standard techniques in the art. The antibody was designated AB-1 (C2).

12. The efficacy of the AB-1 (C2) antibody preparation was assessed against the human MDA-MB231 breast tumour growing as subcutaneous xenografts in female BALB/c *nu/nu* mice. The effect of the antibody preparation was compared with that of the reference therapy, Doxorubicin™ and Vehicle only control.

13. To establish tumors, mice were inoculated with 100 µL of cells ($1x10^7$ cells) injected into the subcutaneous space just below the animal's right shoulder. Thirty female BALB/c *nu/nu* mice that developed tumours from the subcutaneously inoculated MDA-MB231 cells were selected for the study. The mice had an age range of 10-12 weeks and weight range 18.90-23.55g (mean 21.51g) at onset of treatment and were randomly divided in to 3 study groups (2 test and 1 control). Each animal was identified by a transponder (Bar Code Data Systems, Botany Bay, NSW, Australia) that was scanned with a barcode reader (DataMars LabMax I). The transponder was implanted by subcutaneous injection between the shoulder blades while the mouse was under isofluorane-induced anaesthesia.

14. The animals were kept in a controlled environment (targeted ranges: temperature 21±3°C, humidity 30-70%, 10-15 air changes per hour), with a light/dark cycle each of 12 hours, and under barrier (quarantine) conditions.

Temperature and relative humidity were monitored continuously. All animals were subjected to the same environmental conditions and standard diet.

15. The treatment of mice began eight days after MDA-MB231 cell inoculation, when the average tumour volume was 163 mm^3 (average variability of 4.7%).

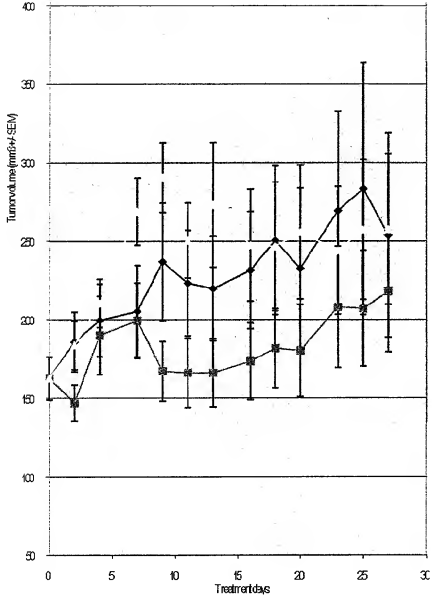
16. The groups were treated with either the Vehicle Control (Phosphate Buffered Saline), AB-1 (C2) (50 mg/kg in sterile PBS) or Doxorubicin™ (1.91 mg/kg in sterile saline). The Vehicle Control and AB-1 (C2) were each administered three times per week by intraperitoneal (i.p.) injection. Doxorubicin™ was administered three times per week by intravenous (i.v.) injection, via the tail vein.

17. Body weight and tumour size measurements were recorded for all animals three times per week, beginning on Day 0, and including the termination day (Day 27).

18. Adverse clinical signs were not associated with the treatments. All groups gained significant mean body weight during the study period.

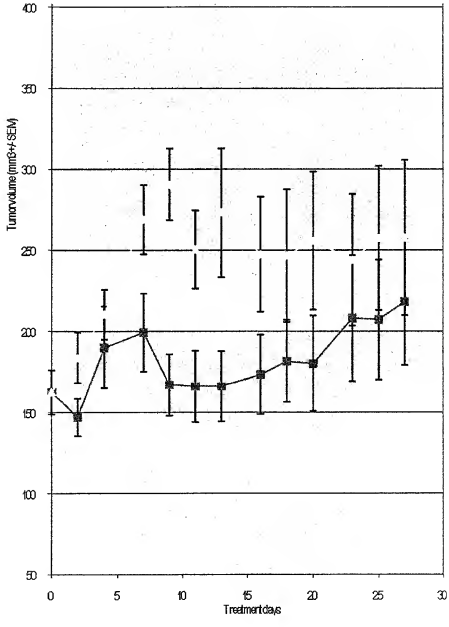
19. FIG. 1 shows the full tumor size dataset for all study groups monitored during the course of the in vivo anticancer experiment. The black diamond shaped points are for the Vehicle Control group (PBS), tan square points are for the AB-1 (C2) group while the lightly shaded triangle shaped points are for the Doxorubicin™ group.

FIGURE 1


400
350
300
250
200
150
100
50
Tumorvolume(mm3+/-SEM)
0
5
10
15
20
25
30
Treatmentdays

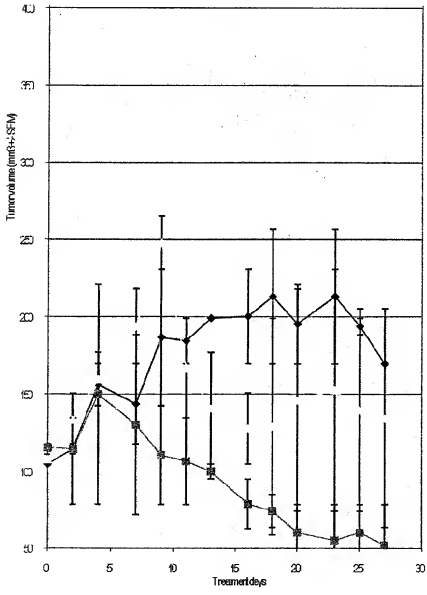
20. FIG. 2 shows the tumour growth data shown in FIG. 1 excluding the Vehicle Control data. The darker quare points are for the AB-1 (C2) group while the lightly shaded triangle shaped points are for the Doxorubicin™ group.

FIGURE 2



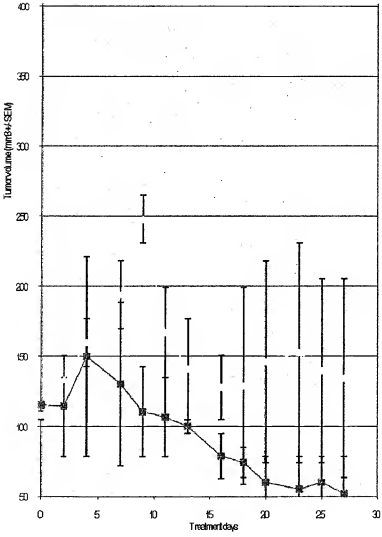
21. FIG. 3 shows the tumour growth data for the two smallest starting tumours in each of the study groups. The dark diamond shaped points are for the Vehicle Control group (PBS), the tan square points are for the AB-1 group (anti-EphB4 antibody) while the lightly shaded triangle shaped points are for the Doxorubicin™ group.

FIGURE 3



22. FIG. 4 shows the tumour growth data shown in FIG. 3 excluding the Vehicle Control data. The darker square points are for the AB-1 (C2) group while the lightly shaded triangle shaped points are for the Doxorubicin™ group.

FIGURE 4



23. As shown in FIG. 1 to FIG. 4, tumour growth inhibition (measured by tumour volume) achieved following treatment with AB-1 (C2) was demonstrated to be superior to that achieved with Doxorubicin™.

24. As can be seen in FIG. 1 and FIG. 3, growth of the tumours in the Vehicle Control group was slower than anticipated. Importantly, however, tumour growth patterns observed with Doxorubicin™ treatment were consistent with previous studies using the MDA-MB231 tumour model at this dose. In contrast, the tumour growth patterns observed in the Vehicle Control group were not consistent with previous studies using the MDA-MB231 tumour model, suggesting that growth of tumours in the Vehicle Control group was compromised. As such, FIG. 2 and FIG. 4 show the data from FIG. 1 and FIG. 3, respectively, with the Vehicle Control group data excluded.

25. In brief summary, thirty female BALB/c *nu/nu* mice, which developed tumours from subcutaneously inoculated MDA-MB231 cells (1 x 107 cells/mouse), were selected for the study. The mice were implanted with a uniquely identified microchip and randomised into three treatment groups of ten mice each. The allocation of mice was such that each group had similar mean starting tumour volumes of approximately 163 mm^3 with a variability of 4.7%.

26. The groups were treated with either Vehicle Control, Phosphate Buffered Saline (PBS), AB-1 (C2) (50 mg/kg) or Doxorubicin™ (1.91 mg/kg). The Vehicle Control and AB-1 (C2) were each administered three times per week by intraperitoneal (i.p.) injection. Doxorubicin™ was administered three times per week by intravenous (i.v.) injection, via the tail vein.

27. Treatments began on Day 0. The study was initially scheduled to continue for three weeks. Due to delayed growth of the tumours in the Vehicle Control group and with the consent of the Sponsor, the treatment period was extended for an additional week.

28. Body weight and tumour size measurements were recorded for all animals three times per week, beginning on Day 0, and including the termination day (Day 27).

29. Adverse clinical signs were not associated with the treatments. All groups gained significant mean body weight during the study period.

30. Tumour growth inhibition achieved following treatment with AB-1 (C2) was demonstrated to be superior to that achieved with Doxorubicin™, however, slower than anticipated growth of the tumours in the Vehicle Control group meant that definitive statistical comparison of the tumour volume data at termination could not be made. Importantly, tumour growth patterns observed with Doxorubicin™ treatment were consistent with previous *vivo*Pharm studies using the MDA-MB231 tumour model at this dose (data not shown in this report). In contrast, tumour growth patterns observed in the Vehicle Control group were not consistent with previous *vivo*Pharm studies using the MDA-MB231 tumour model (data not shown in this report) suggesting that growth of tumours in this group was compromised in some way, the cause and reasons for which are unknown.

Efficacy in Other Cancers

31. As noted by Examiner, the specification demonstrated that EphB4 expression is upregulated in colon and breast cancer cell, and that polyclonal antibodies to EphB4 induced cell death in breast and colon cancer cell lines.

32. The teachings regarding breast and colon cancer cell lines can be extrapolated without undue experimentation to other cancer cells in which EphB4 expression is upregulated. I reach this conclusion because it is the EphB4 epitope that is being targeted and that epitope is common to the EphB4 that is expressed by other cells in other forms of cancers in which EphB4 is upregulated. An increased expression of EphB4 in any tumour suggests that EphB4 signal is playing a role in the development of the tumour phenotype.

33. Thus, by the specification teaching and demonstrating that cell death can be induced in tumour cells such as breast and colon cancer cell lines by targeting

the epitope on EphB4 that is between amino acids 200 and 400 of SEQ ID NO:1, the specification teaches the specific target that is all that the artisan needs to know to also practice this invention against other tumour cells and cancers in which EphB4 is upregulated.

34. The structure of EphB4 that was expressed in the breast cancer cells and colon cancer cells as demonstrated in the examples is the same as, or very similar to, the EphB4 that is expressed in other tumor types.

35. The role or mechanism of action of EphB4 is expected to be the same in other cancer cell types that express EphB4 as it is in breast cancer cells and colon cancer cells.

36. The effect of an antibody that is directed to an epitope between amino acids 200 and 400 of SEQ ID NO:1 would be the same in any cancer cell in which EphB4 is upregulated, including the impact of the same on inhibiting the cancerous growth of the cell, or inducing cell death, or in treating or preventing cancer that results from such cells in the subject.

37. Therefore, since the target is the same, and the same antibodies can be used against the those targets in a variety of cancer cell types, I conclude that the invention can be practiced without undue experimentation in cancers other than those exemplified in the examples of the specification.

38. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,

Date: 27/2/2009